

Vitamin A and the Susceptibility of Respiratory Tract Tissues to Carcinogenic Insult

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The influence of vitamin A on the development of chemically induced lung carcinomas in rats was investigated. Rats were maintained on low, "normal" and excess levels of retinyl acetate (RA). Respiratory tract-squamous carcinomas were induced by intratracheal injections of 3-methylcholanthrene (3-MCA). The carcinogen doses used ranged from 1.25 to 10.0 mg of 3-MCA. Serial sacrifices conducted during the first 20 weeks following carcinogen exposure showed that metaplastic lung nodules, presumed to be precursors of later appearing carcinomas, occurred earlier and at a higher incidence in rats maintained on low levels of RA than in rats maintained on moderate or high levels of RA. The development of invasive pulmonary carcinomas was enhanced at all four carcinogen doses in rats receiving low levels of RA as compared to rats receiving moderate or high levels of RA. No consistent difference in lung cancer incidence existed between the groups receiving normal and high levels of RA. The data clearly show an increased susceptibility of vitamin A-deficient rats to develop chemically induced lung cancers. Possible mechanisms underlying this effect are discussed.

Introduction

The development and maintenance of the normal mucosa of the respiratory tract and of other epithelial tissues is dependent on an adequate supply of vitamin A. Severe vitamin A deficiency leads to abnormal cellular differentiation and proliferation in many epithelial linings. In the respiratory tract (and other mucous membranes) the result is loss of mucociliary epithelium and its replacement by metaplastic squamous epithelium.

In recent years, several laboratories (1-7) including our own, have addressed the question whether vitamin A deficiency alters the susceptibility of mucus membranes to carcinogenic agents. Our previous studies (5) showed that animals maintained on low levels of vitamin A intake at the time of carcinogen exposure develop a greater number of squamous lung nodules, presumed to be the precursors of later appearing squamous carcinomas (6, 7), than animals on high levels of vita-

min A. While this was initially interpreted as a "therapeutic" effect of high vitamin A (retinyl acetate) intake, later studies showed that it was the manifestation of a greater susceptibility to carcinogens of "low intake" animals, which was abolished by high levels of vitamin A. Subsequent studies (7) showed that this state of increased susceptibility to the carcinogen effects could be corrected as late as 10 weeks after exposure to the carcinogen, suggesting that the cellular events during the post-initiation phase of carcinogenesis were enhanced or accelerated by the state of partial Vitamin A deficiency. Finally, we showed that vitamin A, stored in the liver, does not protect animals against developing an increased state of susceptibility to carcinogens (6) when their external vitamin A supply is cut off.

All of these studies were carried out by using the development of lung squamous nodules as an experimental end point. The definitive proof, that vitamin A deficiency results in an increased incidence of pulmonary carcinomas in carcinogen exposed animals, was missing. In the present communication lung cancer induction studies in vitamin A deficient animals are, for the first time, reported. An increased incidence of chemically induced pulmo-

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nary squamous cell carcinomas in animals maintained on low vitamin A levels (but not totally deprived of vitamin A) is described. The results are discussed in the light of recent findings reported from other laboratories, describing the cellular and tissue alterations occurring in Vitamin A deficiency.

Experimental Design

Specific pathogen-free Fischer 344 rats (male and female) were placed on a semisynthetic vitamin A free diet (TD-69389, General Biochemicals) at 3 weeks of age. One week later they were divided into three groups which received different levels of retinyl acetate (RA) supplements twice weekly by gavage, amounting to a weekly dose of 1740, 174, or 17.4 μg RA per rat. Five weeks after the start of the

RA administration, the animals received two intratracheal injections (on consecutive days) of the carcinogenic polycyclic hydrocarbon 3-methylcholanthrene (3-MCA). The total dose of 3-MCA administered per animal was 10, 5, 2.5, or 1.25 mg of carcinogen. Thus, for each dose level of RA, there were four groups of rats receiving different doses of carcinogen. The methodological details have been reported previously by (6, 7). Rats from each group were either killed in a serial sacrifice study to determine the incidence of metaplastic lung nodules as previously described (6, 7), or were used in a survival study to establish final lung tumor incidences.

Results

Development of Metaplastic Lung Nodules

Nearly 200 rats were killed in a serial sacrifice study to establish the time of appearance and the incidence of metaplastic lung nodules, which are considered to be precursors of later appearing carcinomas, though not every metaplastic nodule necessarily develops into a carcinoma (5) (also compare Tables 1 and 3.) As seen in Table 1, the metaplastic lung nodule response shows definite dose- and time-related trends, with the greatest number of positive animals at 10 mg 3-MCA at 20 weeks. Table 2 shows that the development of the metaplastic lesions is enhanced in those animals receiving subnormal levels of vitamin A, compared to rats maintained on normal to high vitamin A levels.

Development of Respiratory Tract Carcinomas

More than 230 rats (15–22 rats per subgroup) were used in a survival study to determine the lung tumor incidence in the groups maintained on different levels of vitamin A. Animals were killed when

Table 1. Effect of 3-methylcholanthrene (3-MCA) on the incidence of metaplastic lung nodules in rats."

Dose 3-MCA, mg	Rats with lung nodules after 3-MCA injection, %	
	10 weeks	20 weeks
10	33	74
5	31	56
2.5	4	33
1.3	17	30

" Data based on 18–27 rats per group and time point. Animals from subgroups maintained at different retinyl acetate levels are pooled.

Table 2. Effect of retinyl acetate (RA) on the incidence of metaplastic lung nodules in rats."

Dose RA/week, μg	Rats with lung nodules after 3-MCA injection, %	
	10 weeks	20 weeks
1744.0	0	47
174.0	7	33
17.4	58	64

" Data based on 24–36 rats per group and time point. Rats from four different carcinogen subgroups are pooled for each RA level.

Table 3. Effect of retinyl acetate (RA) on the incidence of squamous cell carcinomas in rats."

Dose RA/week, μg	Carcinogen dose								TBA, % combined ^d
	10 mg		5 mg		2.5 mg		1.3 mg		
	TBA, % ^b	MST, week ^c	TBA, % ^b	MST, week ^c	TBA, % ^b	MST, week ^c	TBA, % ^b	MST, week ^c	
1744.0	66	77	20	104	9	110	10	111	24
174.0	40	85	21	100	10	105	0	112	16
17.4	93	70	65	82	27	101	23	103	48

" Each of the 12 subgroups consists of 15–22 "effective" animals (233 rats total). 3-MCA was administered intratracheally; the RA by gavage.

^b TBA, tumor-bearing animals. All tumors were invasive squamous carcinomas.

^c MST = mean survival time.

^d For each RA level, % TBA of all carcinogen dose groups were combined; there were 77–79 rats per RA level.

moribund or at 150 weeks after intratracheal injection of carcinogen. Complete autopsies were performed on all animals. Histological slides were prepared from liver, spleen, kidney, and lung, as well as all other organs showing grossly visible abnormalities. The details of the procedures used in this study have been previously described (6, 7). The results of the lung tumor induction study (Table 3) showed that rats receiving a low level of RA supplement ($17.4 \mu\text{g}/\text{wk}/\text{rat}$) have a higher lung cancer incidence than rats receiving a medium or high (174 or $1774 \mu\text{g}$) RA supplement. This was observed at all four carcinogen dose levels but was most pronounced at the two medium carcinogen doses (5 mg and 2.5 mg of 3-MCA per rat). In these two groups, an approximately 3-fold difference in lung cancer incidence was noted between the lowest and the medium to high RA groups. It is also noteworthy that the incidence of metastases from lung cancers was twice as high in the low RA dose group as compared to the medium and high RA dose groups (not shown). All diagnosed lung tumors were invasive squamous cell carcinomas. No effect on the development of other tumors (genital tract, mammary, pituitary and lymphoreticular tumors) was noted. This is possibly due to the low incidence in each tumor category in the individual groups, as well as the "competing risks" between the different tumor types which tend to obscure tumor incidence data in long term survival studies. The effect of vitamin A on lung cancer induction is also reflected in the mean survival times, which are shortest in the animals maintained on the low RA level. A reduction in survival is most clearly seen in the groups of rats having been exposed to an intratracheal dose of 5 mg 3-MCA. In this group, the mean survival time of the low RA maintenance group was approximately 20 weeks shorter than that of the medium and high RA groups (Table 3 and Fig. 1).

The vitamin A levels in liver and serum of control rats maintained on the 3 different RA supplements for 126 weeks is summarized in Table 4. At that time, the liver vitamin A storage was below detectable levels in the low RA maintenance group, approximately $0.04 \mu\text{g}/\text{mg}$ liver in the medium RA maintenance group, and $3.56 \mu\text{g}/\text{mg}$ liver in the high RA maintenance group. Rats maintained for 1 year on regular laboratory diet, Purina 5010C (approx. $4 \mu\text{g}$ retinyl esters/g diet after pasteurization) have about $1.3 \mu\text{g}$ of vitamin A/mg of liver.

Discussion

The data presented clearly indicate that rats maintained on a low vitamin A intake are more susceptible to lung cancer induction by carcinogenic

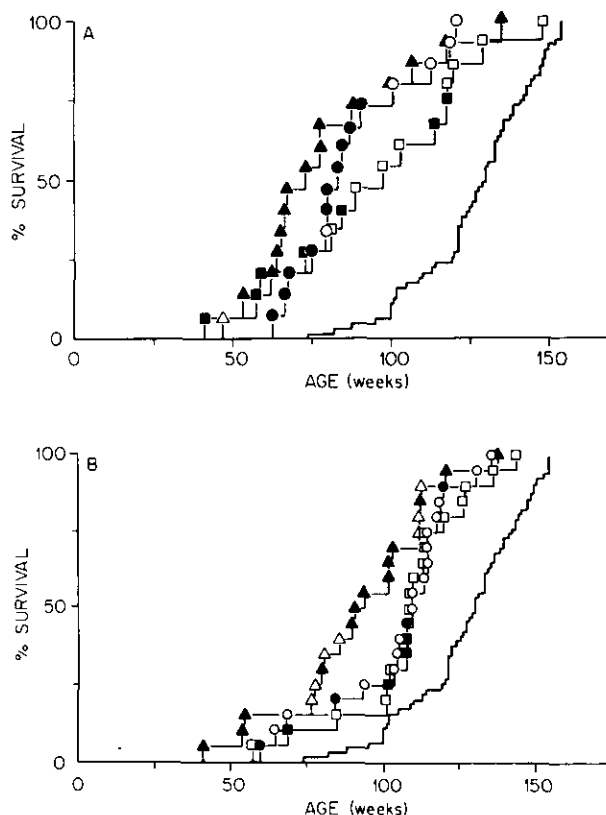


FIGURE 1. Effect of retinyl acetate on survival of groups of rats intratracheally exposed to carcinogen: (O, ○) $1744 \mu\text{g}$ RA/wk; (□, ■) $174 \mu\text{g}$ RA/wk; (△, △) $17.4 \mu\text{g}$ RA/wk; (—) survival of rats on normal lab chow; (●, ■, ▲) animals died with lung carcinoma; (○, □, △) animals died without lung cancer. (A) Groups of 15-22 rats which received 10 mg 3-MCA intratracheally, 5 weeks after start of RA administration; (B) groups of 15-22 rats which received 5 mg 3-MCA intratracheally, 5 weeks after start of RA administration.

Table 4. Liver and serum vitamin A levels of F-344 rats maintained on different retinyl acetate (RA) supplement.*

Dose RA/week, μg	Retinyl ester	
	In liver, $\mu\text{g}/\text{mg}$	In serum, $\mu\text{g}/100 \text{ ml}$
1744.0	3.56	56.3
174.0	0.04	34.6
17.4	0.00	14.5

* Two to four rats were killed 24 hr after the last intragastric intubation. Rats received vitamin A-free diet and RA supplement as indicated for 126 weeks.

polycyclic hydrocarbons than rats maintained on an adequate or high vitamin A intake [approximately $100 \mu\text{g}$ of RA/week is considered adequate dietary intake (3)]. This increased susceptibility of vitamin A deficient rats (not totally vitamin A-deprived) to lung cancer induction is manifested in a higher lung

cancer incidence and a shorter survival (due to death from lung cancer), compared to rats receiving adequate or high levels of the vitamin. The other important finding is that RA intake 10 times in excess of an "adequate" level does not appear to confer any added protection.

These results support our previous conclusions (6, 7) which were based on studies with carcinogen-induced metaplastic lung nodules. Until now, the conclusive evidence for an enhanced lung cancer development in vitamin A deficiency was missing.

There is at least suggestive evidence that vitamin A deficiency also enhances carcinogenesis in other organ systems (3). However, conflicting results have been obtained with different types of carcinogens, i.e., direct acting versus metabolically activated carcinogen (1). Recent epidemiological evidence (8) suggests that in humans, a low dietary intake of vitamin A or closely related dietary factors is associated with an increased lung cancer risk.

The available data, though still scanty, raise the question as to a possible mechanism by which vitamin A deficiency might enhance carcinogenesis in certain epithelial tissues. Studies of Genta et al. (4) indicate that covalent binding of the polycyclic hydrocarbon benzo[a]pyrene to DNA of tracheal epithelium is enhanced during vitamin A deficiency. Conversely Hill and Shih (9) have suggested that retinol and some of its derivatives inhibit the formation of epoxides during the metabolism of benzo[a]pyrene. Thus one possible mechanism by which vitamin A deficiency might enhance carcinogenesis is by way of enhanced metabolic activation and/or DNA binding of carcinogen. However, as we have previously shown (7), Vitamin A deficiency also appears to enhance processes during the "post-initiation phase" of carcinogenesis. These findings are in a general way supported by more recent studies by Verma and Boutwell (10), who demonstrated that retinoic acid is a potent inhibitor of crucial biochemical events occurring during promotion of skin carcinogenesis.

Other observations made in the respiratory tract of Vitamin A deficient animals may also be of relevance to this discussion. It has been known for many years that squamous metaplasias develop in the respiratory tract of vitamin A-deficient animals (11-13). More recently, it was shown that more generalized changes in cell distribution and cell proliferation occur in respiratory tract epithelium of deficient animals. In the tracheal epithelium of such animals the relative number of basal cells is increased and the relative number of ciliated cells decreased (14). Labeling index and mitotic index are significantly increased above normal levels (15).

Such tissue changes might in various ways enhance the induction or expression of neoplastic cell populations in the epithelium.

There is evidence indicating that the respiratory tract mucosa of vitamin A-deficient animals is not only more susceptible to carcinogenic chemicals but also to other injurious agents. Bang and his associates (16-19) have shown in a number of studies that the upper respiratory tract mucosa of vitamin A deficient chicks is more susceptible to infection with Newcastle Disease and influenza viruses. Loosli and his colleagues (20, 21) found that the postinfluenza pathology in the lungs of vitamin A deficient mice is more severe and more extensive than in controls, leading to widespread epithelial proliferation and squamous metaplasias in the distal lungs.

Recent studies in our own laboratory have shown that exposure to nitrogen dioxide causes more severe and long-lasting morphological and biochemical abnormalities in the lungs of vitamin A-deficient rats (unpublished observations). The increase in DNA synthesis seen after NO₂ exposure, as well as the increase in phospholipid content and/or synthesis of the lungs, were markedly enhanced and persisted over a longer time span in deficient animals. This suggests that the same NO₂ exposure may indeed be considerably more harmful during a state of relative vitamin A deprivation than during normal dietary intake of vitamin A.

In summary, studies in several laboratories strongly suggest that respiratory tract tissues of vitamin A-deficient animals (and possibly humans) have a heightened susceptibility to a variety of noxious insults. Such injurious agents include carcinogens, infectious viruses, and gaseous air pollutants. The fact that this increased susceptibility may occur not only during complete vitamin A deprivation of the organism but also during temporary reduction of intake gives the findings added significance. It appears that far more effort is needed to study the interactions of dietary effects and environmental injuries before the possible impact of such synergisms on human health can be assessed.

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